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MONOCLONAL ANTIBODY

Anti-Mouse Integrin α7

Code No.	Clone	Subclass	Quantity	Concentration
K0046-3	3C12	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: The integrin family of adhesion molecules participate in important cell-cell and cell-extracellular matrix interactions in a diverse range of biological processes. Integrins are heterodimers consisting of an α subunit and a β subunit. Both α and β subunits are transmembrane proteins with large extracellular domains (>100 kDa for α subunit and >75 kDa for β subunit) that interact with extracellular matrix proteins and relatively small cytoplasmic domains (50 amino acids or less, except for the β4 subunit) that interact with cytoskeletal proteins. The adhesiveness of integrins is dynamically regulated in response to cytoplasmic signals, termed "inside-out" signaling. It has been reported that, upon ligand binding, integrins regulate many intracellular signaling pathways that involve cytoplasmic alkalization, intracellular Ca^{2+} fluctuation, inositol lipid metabolism, protein kinase C, MAP kinase and phosphatidyl inositol kinase. Integrin α7 is a specific cellular receptor for the basement membrane protein laminin-1, as well as for the laminin isoforms-2 and -4. The α7 subunit is expressed mainly in skeletal and cardiac muscle and may be involved in differentiation and migration processes during myogenesis. Absence of integrin α7 results in muscular dystrophy is revealed.

SOURCE: This antibody was purified from hybridoma using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Integrin α7 knockout C57/B6 mouse splenocyte immunized with mouse myoblasts.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. Contains no preservatives.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with mouse Integrin α7 on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 10 µg/mL
Flow cytometry; 10-20 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not tested	C2C12	Not Tested
Reactivity on FCM		+	

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

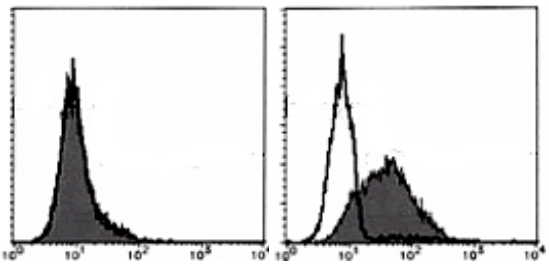
REFERENCES:

1) Samson, T., *et al*., *J. Biol. Chem*. **279**, 28641-28652 (2004)
2) Volpers, C., *et al*., *J. Virol*. **77**, 2093-2104 (2003)
3) Rosbottom, A., *et al*., *J. Immunol*. **169**, 5689-5695 (2002)
4) von der Mark, H., *et al*., *J. Biol. Chem*. **277**, 6012-6016 (2002)
5) Mielenz. D., *et al*., *J. Biol. chem*. **276**, 13417-13426 (2001)

Clone 3C12 is used in these references.

RELATED PRODUCTS:

K0046-5 PE labeled anti-Mouse Integrin α7 (3C12)
K0047-3 Anti-Mouse Integrin α7 (6A11)
K0099-3 Anti-MIBP (5B4.7)
D050-3 Anti-β1-Integrin (CD29) (AG89)
D050-5 PE labeled anti-β1-Integrin (CD29) (AG89)



Flow cytometric analysis of mouse Integrin α7 expression on NIH/3T3 (left) and C2C12 (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of K0046-3 to the cells.*

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Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps after 2).

1) Detach the cells from the culture dish by using cell dissociation buffer (Invitrogen; code no. 13151-014).
2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
3) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
4) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
5) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
6) Add 40 µL of the primary antibody at the concentration suggested in the **APPLICATIONS,** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
8) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
9) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; C2C12)

Immunocytochemistry

1) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
2) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the cultured cells on the glass slide by soaking the slide with plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat wash once more.
3) Add 30 µL of 1:40 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by covering with aluminum foil.
4) Wash the slide in plenty of PBS as in step 2).
5) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
6) Promptly add Permafluor™ aqueous mounting medium (MBL; code no. IM-0752) onto the slide, then put a cover slip on it.

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